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Research Paper

Geospatial and co-occurrence analysis of antibiotics, hormones, and UV filters in the Chesapeake Bay (USA) to confirm inputs from wastewater treatment plants, septic systems, and animal feeding operations

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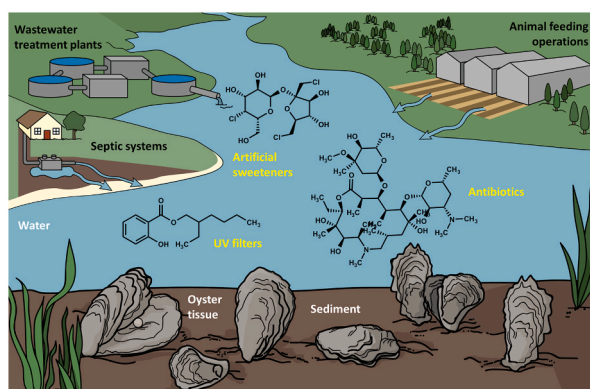
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HIGHLIGHTS

- Sucralose concentrations were higher in more populated regions of the Chesapeake Bay.
- Higher antibiotic levels were found at sites near animal feeding operations.
- Sucralose and hormones were more closely associated with septic systems than WWTPs.
- Octisalate (UV filter) was detected at the highest concentration in sediment and oysters.
- Toxicity thresholds were exceeded for select antibiotics and UV filters.

GRAPHICAL ABSTRACT



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ABSTRACT

Previous studies have reported select contaminants of emerging concern (CECs) in limited areas of the Chesapeake Bay (USA), but no comprehensive efforts have been conducted. In this work, 43 antibiotics, 9 hormones, 11 UV filters, and sucralose, were measured in matched water, sediment, and oyster samples from 58 sites. The highest sucralose concentration was 3051 ng L⁻¹ in a subwatershed with 4.43 million liters of wastewater effluent per day (MLD) and 4385 septic systems. Although antibiotic occurrence was generally low in subwatersheds located in less populated areas, 102 ng L⁻¹ ciprofloxacin was detected downstream of 0.58 MLD wastewater effluent and 10 animal feeding operations. Hormones were not regularly detected in water (2%) or oysters (37%), but the high detection frequencies in sediment (74%) were associated with septic systems. UV filters were ubiquitously detected in oysters, and octisalate exhibited the highest concentration (423 ng g⁻¹). Oyster-phase

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oxybenzone and aqueous-phase sucralose concentrations were significantly correlated to wastewater effluent and septic systems, respectively. Toxicity outcomes were predicted for homosalate and octisalate throughout the Bay, and antimicrobial resistance concerns were noted for the Chester River. The geospatial and co-occurrence relationships constitute crucial advances to understanding CEC occurrence in the Chesapeake Bay and elsewhere.

1. Introduction

Several previous studies have measured contaminants of emerging concern (CECs) in the Chesapeake Bay, which is the largest estuary in the United States [1], but the spatial distribution of CECs in water, sediment, and biota remains largely unknown due to the relatively small areas sampled in those campaigns (Fig. 1). Antibiotics and hormones have been detected in surface water from less populated, rural areas of the Chesapeake Bay [2–5], potentially stemming from animal feeding operations (AFOs) [6] or shallow groundwater discharges [7]. The occurrence of antibiotics, hormones, and personal care products has also been reported in more populated areas of the Bay [4,8,9]. The norfloxacin antibiotic was present at levels up to 94 ng L⁻¹ in the Chester River, which has 3792 upstream septic systems and receives 4.24 million liters of effluent each day (MLD) from upstream wastewater treatment plants (WWTPs) [10]. In contrast, high concentrations of the estrone and

17 β -estradiol hormones were measured in sediment from the mouth of the Manokin River, which drains an agricultural watershed with 14 AFOs that produce over 2 million chickens per year, but these hormones were not detected in the Chester River [10]. The literature suggests different influences of urban and agricultural sources on the concentration and distribution of CECs in the Chesapeake Bay. Here, "source" refers to the entry point of CECs into the environment (e.g., WWTPs, septic systems, AFOs). As previous studies have only focused on a few analytes, phases (i.e., water, sediment, or biota), and/or regions of the Chesapeake Bay, this study seeks to fill knowledge gaps on CEC occurrence and distribution through a comprehensive sampling campaign at sites in subwatersheds with different land-use characteristics and potential sources.

Previous CEC occurrence studies from around the world have mainly focused on sampling areas with one primary source type. For example, Kim et al. analyzed water samples from Korean streams and attributed

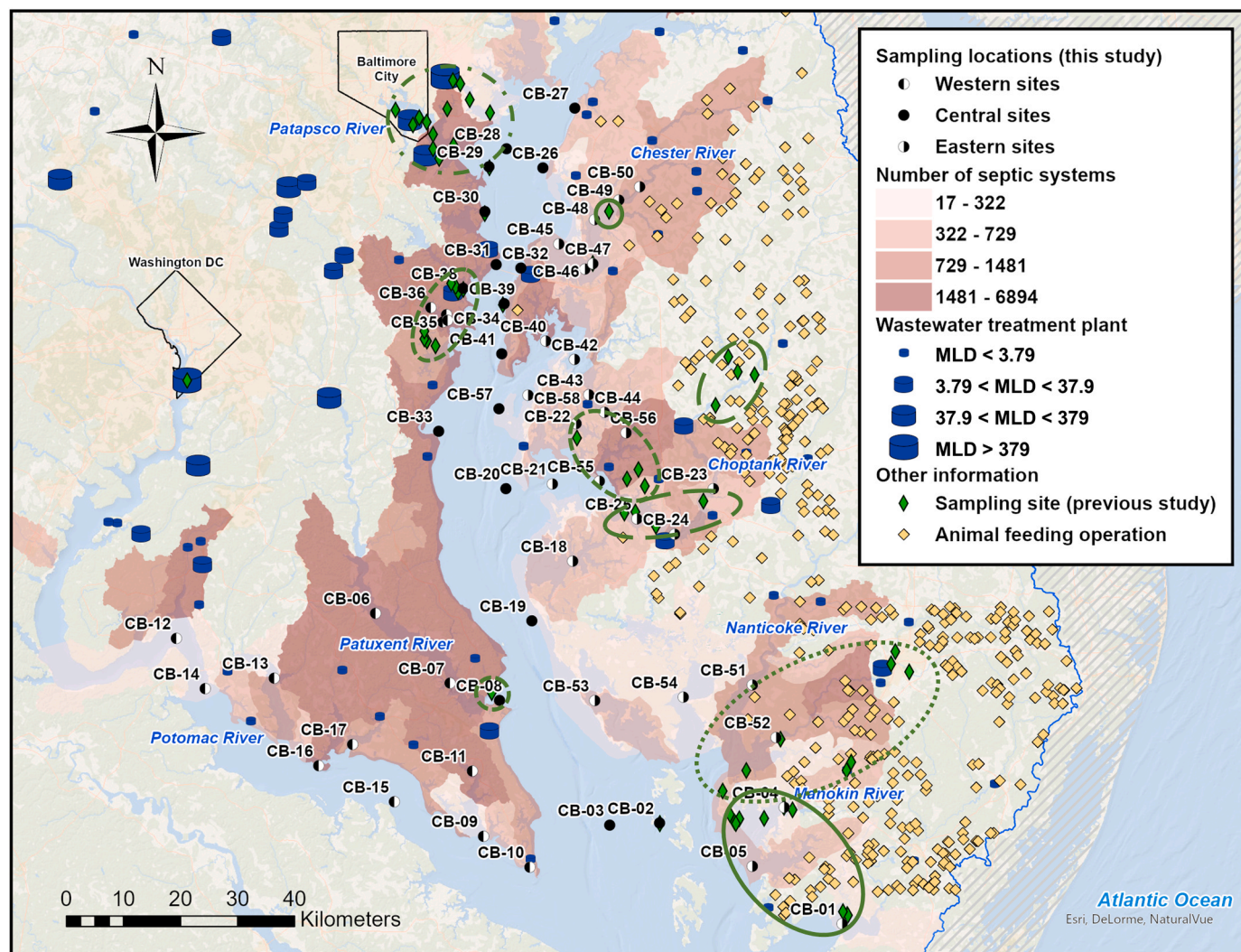


Fig. 1. Sampling sites from this study and previous reports of CEC occurrence in the Chesapeake Bay overlaid with the locations of AFOs [11] and WWTPs [12] and the number of septic systems [13] in each subwatershed. More details on the specific sampling sites, analytes, and phases (e.g., water, sediment, tissue) from previous studies are provided in Table S1. Note, the circled areas correspond to five previous studies (by hatching) to show the limited spatial scale of prior investigations.

the high antibiotic concentrations, including $16.9 \mu\text{g L}^{-1}$ oxytetracycline and $21.3 \mu\text{g L}^{-1}$ sulfamethazine, to AFOs [14]. Maruya et al. examined water, sediment, and fish from the Santa Clara River watershed and Southern California estuaries (USA) and, upon detection of $35 \mu\text{g L}^{-1}$ of the sucralose (artificial sweetener) wastewater indicator, municipal wastewater effluent was implicated as the primary source [15]. Septic systems have been less studied as potential sources of CECs; however, Spoelstra et al. determined that septic systems were the main source of artificial sweeteners in streams from a region of Southern Ontario (Canada) with no WWTPs [16]. The authors also used acesulfame (artificial sweetener) concentrations to estimate that approximately 13% of septic system effluent reaches local streams [16]. Fairbairn et al. investigated the concentrations of 26 CECs in water samples of the mixed land-use Zumbro River watershed (USA) from 2011 to 2012 [17]. The erythromycin and tylosin antibiotics were consistently detected and associated with wastewater, whereas herbicide concentrations were strongly influenced by agricultural land use and season [17]. These studies serve as important confirmations of the potential impacts of different waste(water) sources on CEC levels in the aquatic environment; however, the contributions of multiple source types in mixed land-use watersheds are still widely unknown, especially within the Chesapeake Bay.

Deleterious effects of CECs have been reported for human and ecological health in the Chesapeake Bay watershed. For example, antimicrobial resistance has been documented in *Vibrio* spp., a marine bacteria responsible for most seafood infections [18], at recreational beaches and parks, as well as in commercial fishing and aquaculture areas [19]. Fluoroquinolone-, macrolide-, sulfonamide-, and tetracycline-resistant bacteria have also been measured in the Chesapeake Bay [20,21]. Amato et al. showed that the proportion of antibiotic-resistant *Escherichia coli* isolates was positively associated with poultry AFOs and manure-applied fields [22]. These findings are particularly concerning because bacteria accumulate in Eastern oysters (*Crassostrea virginica*, hereafter "oyster"), which are consumed raw and, thereby, serve as a potential vehicle for multidrug-resistant bacterial infections in humans [23]. Oysters are also a keystone organism in the Chesapeake Bay and provide crucial ecosystem benefits, such as improved water quality and habitat for other species [24]. Previous studies have demonstrated that organic UV filters readily bioaccumulate in aquatic organisms [25,26] and cause oxidative stress [27], hormonal changes [28], and reduced tissue mass [28] in shellfish. While bioaccumulation of UV filters has been reported for rural areas of the Chesapeake Bay [10], the concentrations are expected to be higher in urban and suburban regions that are more heavily impacted by WWTPs and septic systems. Multiple studies have observed endocrine disruption (e.g., lower sperm count, elevated vitellogenin content, higher prevalence of intersex) in fish throughout Chesapeake Bay rivers and tributaries, with the primary sources being hormones and pesticides from wastewater effluent, agricultural runoff, and shallow groundwater discharges [9,29,30].

The objective of this work was to comprehensively investigate the concentrations of 43 antibiotics, 9 hormones, 11 UV filters, and sucralose in matched water, sediment, and oyster samples from 58 sites distributed across the Maryland section of the Chesapeake Bay. The 64 CECs and 58 sites were selected to establish contaminant profiles in regions that are differentially influenced by WWTPs, septic systems, and AFOs. Sucralose was employed as a wastewater indicator to distinguish the influences of anthropogenic (e.g., WWTP, septic system) and agricultural (e.g., AFO) sources [31,32]. The co-occurrence of CECs and the relationships between CEC concentrations and geospatial data from the immediate and upstream subwatersheds were analyzed to confirm sources. Toxicity outcomes of frequently detected CECs were evaluated to identify individual contaminants of concern to Chesapeake Bay organisms and determine specific areas in the Chesapeake Bay with elevated risk.

2. Materials and Methods

2.1. Chemicals

All chemical reagents and standards were obtained from Sigma-Aldrich (St. Louis, MO, USA), Fisher Scientific (Pittsburgh, PA, USA), Toronto Research Chemicals (Toronto, Canada), or CDN Isotopes (Pointe-Claire, Canada). The purity of all chemical standards was at least 95%. Stock and working solutions were prepared yearly and weekly, respectively, according to previously reported protocols [33]. Additional information on the 64 CECs, including the names of individual analytes, their physicochemical properties, and national prescription data for 2017 [34], is summarized in Table S2.

2.2. Sample collection

Water, sediment, and oysters were collected from 58 sites in the Chesapeake Bay between 10 October 2017 and 29 November 2017 using previously reported sampling protocols and best practices for quality assurance and quality control [10]. The sampling locations are shown in Fig. 1, and the site codes, coordinates, baseline water quality parameters (e.g., pH, temperature, salinity), and water depth are available in Table S3. A YSI multimeter (Yellow Springs, OH, USA) was employed to record temperature and salinity, and a fathometer was used to determine water depth. The only variation from our previous protocols was that water was collected from approximately 1 m above the sediment-water interface using a Wildco Kemmerer device (Yulee, FL, USA), rather than just below the air-water interface [10], which is more likely to be influenced by photochemical degradation processes. Sediment and oysters were collected by stainless steel dredge. Sediment was not collected at 35/58 sites due to logistical challenges. Oysters could not be found at site CB-32 and were, therefore, not collected at this location.

2.3. Geospatial data

The 12-digit hydrologic unit code (HUC-12) boundaries were identified from the National Hydrography Dataset Plus High Resolution [35] and will henceforth be referred to as subwatersheds. The hydrodynamics in the main channel of the Chesapeake Bay (i.e., the Upper Chesapeake Bay subwatershed) are inherently different than at sampling sites located in tributaries and coastal embayments [36]. Therefore, sampling sites within the main channel were designated as the "Central" region and excluded from geospatial comparisons on the subwatershed scale. The other sites were classified as "Western" or "Eastern", depending on their location relative to the Central section. The Western region consisted of more urban and suburban land use with a median subwatershed population density of $13.4 \text{ people km}^{-2}$. More agricultural activity occurs in the Eastern section, which had a median subwatershed population density of $7.1 \text{ people km}^{-2}$. The subwatershed and region designations for each site are provided in Table S3. Geospatial data, including the number and total capacity of WWTPs [12], number of AFOs and animals produced [11], number of septic systems [13], and human population [37], were compiled for the immediate and upstream subwatersheds (Table S4). Upstream subwatersheds were defined as adjacent subwatersheds that were hydrologically connected to the subwatershed containing the sampling site (i.e., the immediate subwatershed). Since long-distance fate and transport of CECs are not well-understood in the region, geospatial data were restricted to the State of Maryland, where all sites were located.

2.4. Analytical methods

Sucralose is a conservative tracer that primarily exists in the aqueous phase. The fluoroquinolone ($\log D < 0.88$, $n = 17$), sulfonamide ($\log D < 1.03$, $n = 13$), and tetracycline ($\log D < -3.51$, $n = 8$) antibiotics are

hydrophilic (Table S2) and were not expected to accumulate in sediment or tissue at appreciable levels. One macrolide, clarithromycin, exhibited a log D greater than 2.0, which is a common threshold for hydrophobicity. Nevertheless, 42 out of 43 antibiotics were below this threshold. For this reason, antibiotics and sucralose were only measured in water samples; however, we acknowledge that some antibiotics may accumulate in sediment and tissue [38]. Hormones and UV filters were analyzed in all water, sediment, and oyster samples.

The sample pretreatment, analyte extraction, and quality assurance and quality control protocols were identical to those reported in previous studies [10,33]. Briefly, water samples from each site were split into six 100-mL subsamples, of which three were not modified and three were spiked with 1–100 ng of each analyte for standard additions analysis to measure CEC recovery. The water samples were processed by solid-phase extraction (SPE) with hydrophilic-lipophilic balanced (HLB) cartridges (6 cm³, 150 mg; Waters Corp.; Milford, MA, USA). Freeze-dried sediment and whole oyster tissue samples were also split into six subsamples containing 500 mg and 50 mg, respectively. The solid samples were processed by a modified QuEChERS protocol with reverse-SPE cleanup by HLB cartridges (3 cm³, 50 mg). All extracts were evaporated under nitrogen gas and then reconstituted to 1 mL with 50% methanol containing 10 µg L⁻¹ internal standards.

Analyte concentrations in the reconstituted extracts were measured by liquid chromatography with triple quadrupole tandem mass spectrometry (UltiMate 3000 with Thermo TSQ Quantum Access Max; Waltham, MA, USA). The operating conditions were identical to those reported in a previous study [33]. Method detection limits (MDLs) were calculated as 3 × the signal-to-noise ratio determined from the average of blank solutions [39]. Method quantitation limits (MQLs) were calculated as 3.3 × the corresponding MDLs. When the measured response was below MQL or MDL, the CEC was considered "detected" or "not detected", respectively. Absolute analyte recovery was calculated by standard additions [39], and the average recovery efficiencies are reported in Table S5, along with the MDL and MQL for each analyte. The relative standard deviation on the recovery efficiency was below 20% for all analytes, in accordance with standard recommendations [40]. All concentrations were reported as mean ± standard deviation, and all sediment- and tissue-phase concentrations were reported as dry weight.

2.5. Statistical analyses

All statistical analyses were performed in OriginPro 2016 (Northampton, MA, USA). A one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference test was conducted to compare analyte concentrations between the Western, Central, and Eastern sites. For CECs with at least ten quantifiable detections, Spearman correlation coefficients (ρ) were calculated to explore relationships between (i) CEC concentrations and geospatial data at the immediate and upstream subwatershed scales and (ii) CEC concentrations in the water, sediment, and oyster phases. Differences (ANOVA) and correlations (Spearman) were considered significant when $p < 0.05$.

2.6. Risk assessment

CECs that were detected in water from more than 25% of the surveyed sites in each section of the Bay were considered for risk assessment. The predicted no-effect concentrations (PNECs) of CECs were calculated by dividing the (i) half-maximal effective concentration or half-maximal lethal concentration [41–47] by a standard assessment factor of 1000 or (ii) no-observed effect concentration by a standard assessment factor of 100 [48,49]. The toxicity data used for PNEC calculations are reported in Table S6. The PNECs were compared to aqueous CEC concentrations to evaluate potential risks to aquatic organisms in the Chesapeake Bay.

3. Results and discussion

3.1. Higher sucralose concentrations detected in urban subwatersheds

To establish a foundation of human-derived wastewater in the Chesapeake Bay, we measured concentrations of the artificial sweetener, sucralose, at all study sites. The measured sucralose concentrations are reported in Fig. 2; note, the raw data are available in Table S7. The median sucralose concentrations in the Western, Central, and Eastern regions of the Chesapeake Bay were 634, 674, and 352 ng L⁻¹, respectively (Fig. 3), and significant differences were observed between the Western and Eastern ($p < 0.001$) and Central and Eastern ($p = 0.020$) sections. The highest overall sucralose concentration, 3051 ng L⁻¹, was measured at a site in the Gambo Creek-Potomac River subwatershed, which has four upstream WWTPs that discharge 4.43 MLD along with 4385 septic systems. The range of measured sucralose concentrations (87–3051 ng L⁻¹) was similar to previous reports from other regional locations. For example, Bean et al. detected 354–1364 ng L⁻¹ of sucralose in the Delaware River and Delaware Bay [50]. Asteggianti and Giorgina reported sucralose concentrations of 309–461 ng L⁻¹ in the upper Choptank River [51], in good agreement with the < 3–441 ng L⁻¹ sucralose measured in the lower Choptank River in this study. Given its use as an artificial sweetener, the higher sucralose concentrations in the more populated Western subwatersheds were expected and provided a baseline for wastewater inputs throughout the study area.

Sucralose concentrations were more variable in the Western section, as evidenced by the wider interquartile range (526–1198 ng L⁻¹) compared to the Central (614–876 ng L⁻¹) and Eastern (308–441 ng L⁻¹) regions. The high 75th percentile concentration in the Western section suggested an influence from wastewater effluent and/or septic system discharges at sites in more populated areas. To resolve the influence of each source on sucralose concentrations, the WWTP capacities and number of septic systems were identified for each subwatershed. The sucralose concentrations were significantly, positively correlated to the number of septic systems ($\rho = 0.61$, $p < 0.001$) but not wastewater effluent (as MLD, here and below; $\rho = 0.32$, $p = 0.09$) in the immediate and upstream subwatersheds (Fig. S1). Wastewater is generally considered to be the primary source of sucralose in the environment [52], but few studies have addressed the contributions of septic systems, potentially resulting in misattribution of these CEC sources that are prevalent in low population-density areas [16].

The Western and Eastern sections of the Bay have a total of 45,837 and 26,853 septic systems, respectively (Table S4). For the standard household of 2.8 people, septic systems are expected to produce 757 liters of wastewater per day [53]. Based on that usage, the Western and Eastern sections of the Bay are potentially exposed to 34.8 and 20.4 MLD of wastewater from septic systems, respectively, comparable to the 44.3 and 28.4 MLD from WWTP effluent, respectively. This situation is compounded by the fact that CECs are not degraded as effectively in septic systems as in conventional WWTPs [54,55]. For example, the removal efficiencies of erythromycin and sucralose in a typical activated sludge WWTP were 81% and 27%, respectively, whereas septic systems only achieved 20% and 13% removal, respectively [54].

The sampling sites in the Eastern region of the Chesapeake Bay received less wastewater effluent from upstream sources (Table S4), likely explaining the lower sucralose concentrations and narrower interquartile range. Due to the lower WWTP coverage on the Eastern side of the Bay, septic systems may serve as a primary source of sucralose. Importantly, previous studies have reported sucralose concentrations of 29,000 ± 6000 ng L⁻¹ and 40,000 ± 2300 ng L⁻¹ in wastewater effluent and septic tanks, respectively [56]. Data from select sites without upstream WWTPs bolster the hypothesis that septic systems are important sources. For example, 390 ng L⁻¹ sucralose was detected at the CB-18 site located in the Slaughter Creek-Little Choptank River subwatershed, which contains 422 septic systems and no WWTPs. Given the absence of other sources in this subwatershed and the previously

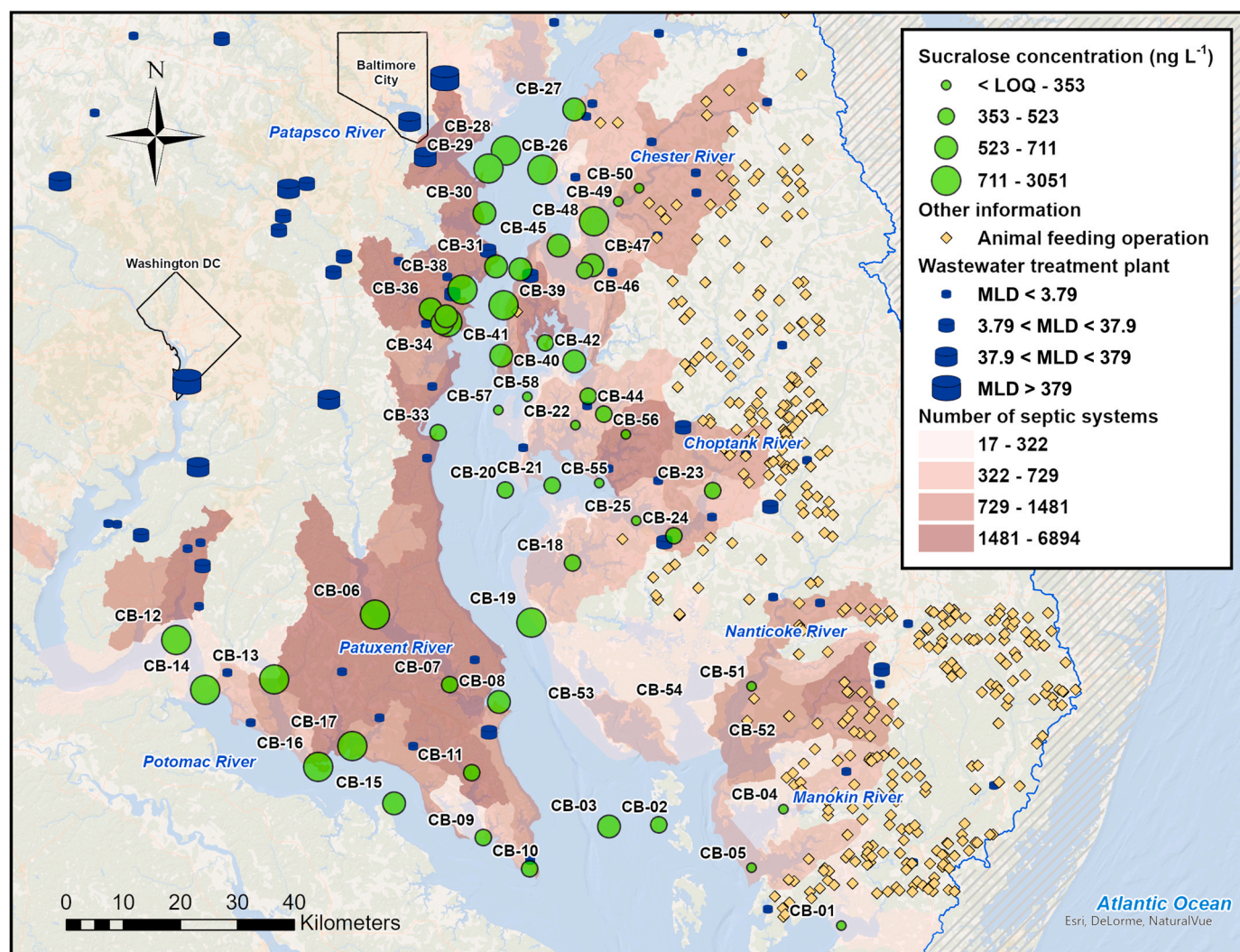


Fig. 2. Sucralose concentrations at each site. The AFO [11], WWTP [12], and septic system [13] data were collected from the Maryland Department of Environment and Maryland Department of Natural Resources.

reported correlation between sucralose concentrations and the number of septic systems (Fig. S1), septic systems are the most likely source of sucralose. Septic systems are also known to influence nutrient [57] and heavy metal [58] levels in the Chesapeake Bay watershed. These results confirm the importance of monitoring for other CECs in areas that only contain non-sewered sanitation systems [57–59].

3.2. Higher antibiotic concentrations measured in rural subwatersheds

The concentrations of individual antibiotics measured at each site are reported in Table S7. In Fig. 4, the total mass concentrations of antibiotics are plotted across the study area. In general, higher levels of antibiotics were present at sites in the upper section of the Bay. The macrolide antibiotics, which included azithromycin, clarithromycin, erythromycin, roxithromycin, and tylosin, exhibited the highest detection frequency by class, but the total concentrations were generally lower than 25 ng L⁻¹. Azithromycin, for which 12.7 M national prescriptions were filled in 2017 [34], was the most frequently detected antibiotic (53/58 sites), and concentrations ranged from below MQL (13 sites, all regions) to 21.5 ng L⁻¹ (CB-49, Eastern region). Three sites in the Chester River, namely CB-46, CB-47, and CB-49, exhibited azithromycin concentrations that exceeded the 19 ng L⁻¹ PNEC reported by Le Page et al. [60]. These sites were located in a subwatershed with three upstream WWTPs discharging a combined 4.24 MLD of effluent.

Previous reports have confirmed that azithromycin is frequently detected in raw wastewater and passes through WWTPs with minimal change in concentration [61], suggesting wastewater as a potential source.

Fig. 5 shows that significant differences in azithromycin concentrations were observed between the Western and Eastern ($p = 0.029$) and Central and Eastern ($p = 0.038$) regions, like sucralose. Unlike sucralose, azithromycin concentrations were higher in the Eastern region. Azithromycin is approved for humans [62] but also widely used in animals [63], potentially explaining the higher concentrations in the Eastern section of the Bay, which contains more AFOs. Clarithromycin, erythromycin, and roxithromycin were only present at low concentrations (i.e., less than 2 ng L⁻¹), but the detection frequencies were higher in the Western region (Fig. 5). Although national prescription data were not available for clarithromycin or roxithromycin, the number of erythromycin prescriptions (2.4 M [34]) filled in 2017 was much lower than for azithromycin (12.7 M [34]) and likely resulted in the lower observed concentrations. Pait et al. attributed erythromycin detections in the Patuxent River and Back River to WWTPs [8], and the same conclusion is proposed in this study. These data confirmed the trace, but consistent, presence of macrolides throughout the Chesapeake Bay and suggest that azithromycin is most likely to exert toxicity outcomes, especially in the Chester River.

The maximum antibiotic concentration measured in this study was 102 ng L⁻¹ of ciprofloxacin at CB-50, which was the most upstream site

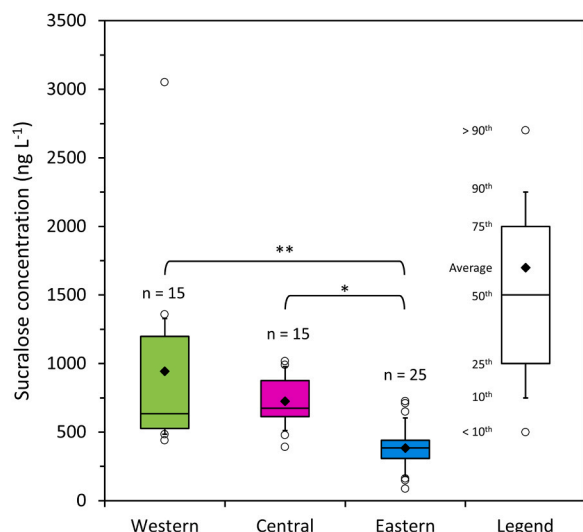


Fig. 3. The concentration distributions for sucralose at sites located in the Western, Central, and Eastern regions of the Chesapeake Bay. The numbered labels in the legend indicate percentiles, the diamond shows the average concentration, and the hollow circles represent concentrations below the 10th and above the 90th percentiles. The median sucralose concentrations were significantly different between select regions (one-way ANOVA), with the * and ** labels indicating $p < 0.05$ and $p < 0.001$, respectively, by Tukey's honest significant difference test.

in the Chester River and downstream of two WWTPs (2.73 MLD) [12] and 3355 septic systems. Importantly, the measured ciprofloxacin concentration exceeded the antimicrobial resistance PNEC of 100 ng L^{-1} [64], suggesting potential selection of antibiotic resistant bacteria in the Chester River. In 2017, ciprofloxacin was the 125th most prescribed medication in the United States with 6.1 M prescriptions filled [34], suggesting that this critically important antibiotic may have been introduced via wastewater effluent or septic systems [65,66]. However, ciprofloxacin is also known to be a transformation product of enrofloxacin [67,68], which is approved for and widely used in poultry [62]. The subwatersheds upstream of CB-50 contain ten AFOs that raise over one million chickens each year. Given the low detection frequency of ciprofloxacin at sites located in the Western and Central sections of the Chesapeake Bay, which are more likely to be impacted by wastewater and less likely to be influenced by poultry operations, the presence of ciprofloxacin in the Eastern region likely derives from enrofloxacin use in poultry [67,69]. The human-use fluoroquinolones, ofloxacin and moxifloxacin, were detected below their MQLs (*i.e.*, 2 and 5 ng L^{-1} , respectively) at 2/58 and 4/58 sites, respectively. Only one detection of moxifloxacin occurred near a WWTP (CB-24, Eastern region), but the other detections were not located near any apparent point sources and did not co-occur with ciprofloxacin. These data suggest that septic systems may be responsible for the ofloxacin and moxifloxacin detections and provide further evidence that ciprofloxacin originated from agricultural sources.

Tetracycline and sulfonamide antibiotics were infrequently identified in Chesapeake Bay water samples. Sulfamethoxazole was found at four sites in the Eastern region, but the concentrations were below MQL (*i.e.*, 5 ng L^{-1}); no other sulfonamides were detected. In 2017, 6.2 M prescriptions were filled in the United States for sulfamethoxazole [34], more than any other sulfonamide. Previous studies have also reported low detection frequencies and concentrations of sulfonamide antibiotics in select areas of the Chesapeake Bay [2,4,10]. For example, He et al. measured 14.8 ng L^{-1} of sulfamethoxazole in the Chester River [10]. Doxycycline, for which 6.7 M prescriptions were filled in 2017 [34], was the only confirmed tetracycline, with concentrations ranging from below MQL (multiple sites) to 39.8 ng L^{-1} (CB-45, Eastern region). These

levels are well below the $2 \mu\text{g L}^{-1}$ minimum selective concentration for antimicrobial resistance [70]. Unlike the other antibiotics, the average concentrations of doxycycline were similar in the Western, Central, and Eastern regions; however, the detection frequency was notably lower in the Eastern section (Fig. 5). Because doxycycline is a common treatment for bacterial infections [71] and acne [72], the higher detection frequencies in the Western and Central sections may stem from the greater upstream populations in these areas. While doxycycline is only prescribed for human use [34], chlortetracycline and oxytetracycline are employed in poultry AFOs [62]. The presence of doxycycline and the absence of animal-use tetracyclines was inconsistent with results from Arikian et al.'s 2005 study, in which 7 ng L^{-1} of oxytetracycline was measured at two sites near CB-24 and CB-25 in the Choptank River (Eastern region), but chlortetracycline, doxycycline, and tetracycline were not detected [2]. The conflicting results may stem from changes in antibiotic use over time [73], especially given the recent emphasis on antibiotic-free animal products.

3.3. Hormones were infrequently detected but generally associated with urban land use

Only one estrogenic hormone, namely 17α -ethinylestradiol, was detected in Chesapeake Bay water. This synthetic compound, which is the active ingredient in birth control [74] and was prescribed 49 M times in 2017 [34], was detected below MQL (*i.e.*, 10 ng L^{-1}) at the CB-51 site near the mouth of the Nanticoke River in the Eastern region (Table S7). The subwatershed containing CB-51 receives 0.24 MLD of wastewater effluent and has 2413 septic systems in the immediate and upstream subwatersheds. The naturally occurring estrogenic hormones, estradiol and estrone, were only present in sediment and oyster tissue. Estradiol was detected below MQL in 2/23 sediment samples, and estrone was measured at 17/23 sites with an average concentration of 6.98 ng g^{-1} . The estrone detection frequency in sediment was higher in the Western (100%) and Central (100%) regions than in the Eastern section (54%) (Table S8). These data agree with findings from Blazer et al., who indicated that urbanized adjacent land use corresponded to higher estrogenicity in terrestrial headwater streams of the Chesapeake Bay [75]. Compared to sediment, estrogenic hormone concentrations were higher in oyster tissue but less frequently detected (Table S9). The maximum estrone concentration of 74.7 ng g^{-1} was found in oysters from CB-13, a site that also exhibited an above-average sucralose concentration (947 ng L^{-1}) for the Western region and confirmed detections of four macrolide antibiotics. The subwatershed containing CB-13 does not include any immediate or upstream WWTPs but does contain 3747 septic systems. Taken together, these findings indicate a substantial influence of septic systems on CEC concentrations at this site.

The low detection frequencies for hormones suggested minimal persistence in the environment; however, previous researchers have detected estrogens and/or estrogenic activity in various regions of the Chesapeake Bay [3,7,30,76]. Furthermore, harmful impacts of estrogenic contaminants have been characterized in large- and small-mouth bass, for which a PNEC of 0.73 ng L^{-1} has been reported for estradiol [9,30,75,77]. Although estradiol was not detected in water samples from this study, the multiple detections of estrogenic hormones in sediment and oyster tissue suggest consistent exposure in areas with WWTPs or septic systems.

3.4. UV filter concentrations were similar throughout the Chesapeake Bay

The site-by-site concentrations of UV filters in water, sediment, and oyster tissue are available in Tables S7, S8, and S9, respectively. Fig. 6 shows that the average UV filter concentrations and detection frequencies were generally similar in all three regions of the Chesapeake Bay. For example, the average aqueous-phase oxybenzone concentrations were 24.7 ± 2.9 , 26.3 ± 4.3 , and $29.8 \pm 2.1 \text{ ng L}^{-1}$ in the Western, Central, and Eastern sections, respectively. Oxybenzone concentrations

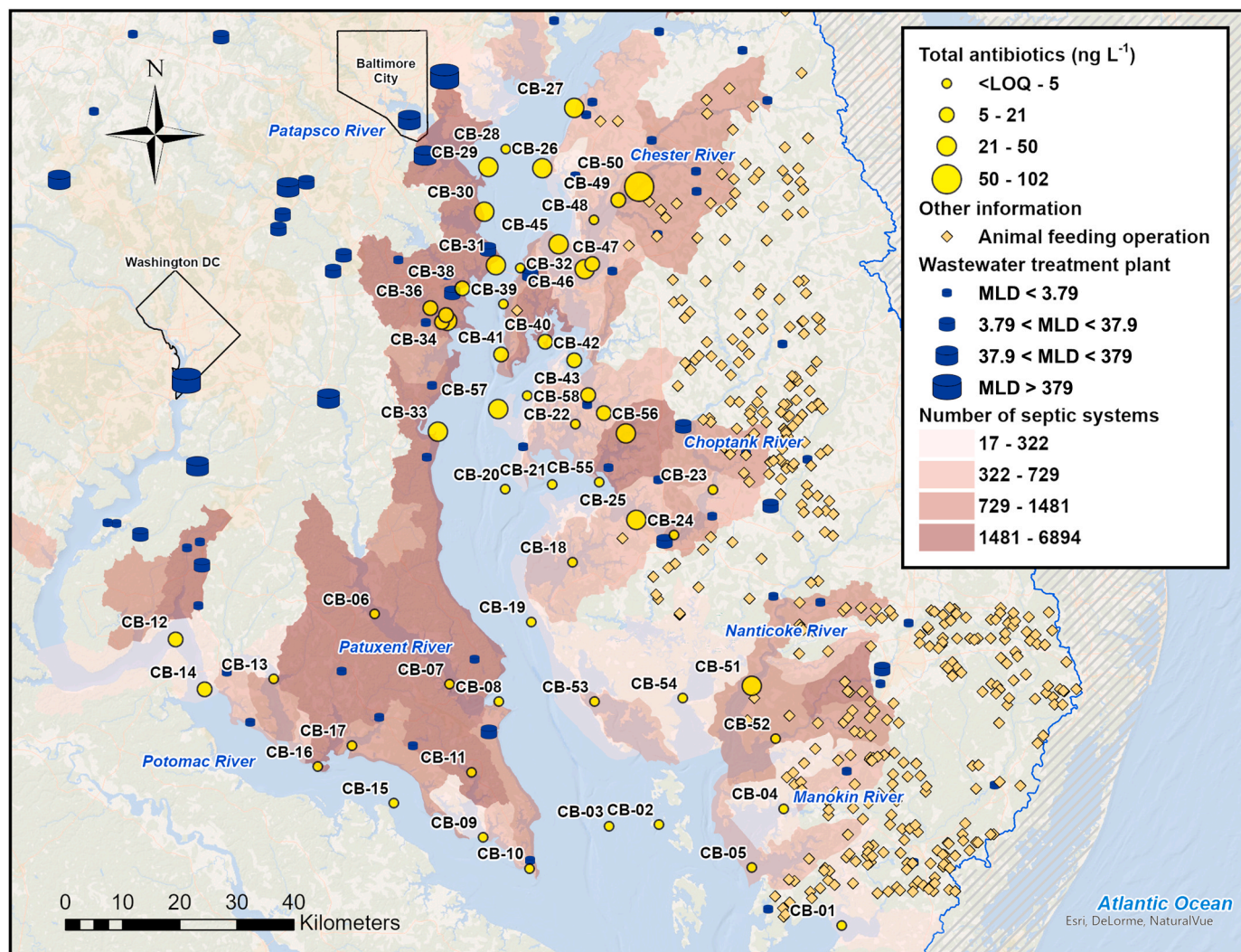


Fig. 4. Total mass concentrations of antibiotics at each site. The AFO [11], WWTP [12], and septic system [13] data were collected from the Maryland Department of Environment and Maryland Department of Natural Resources.

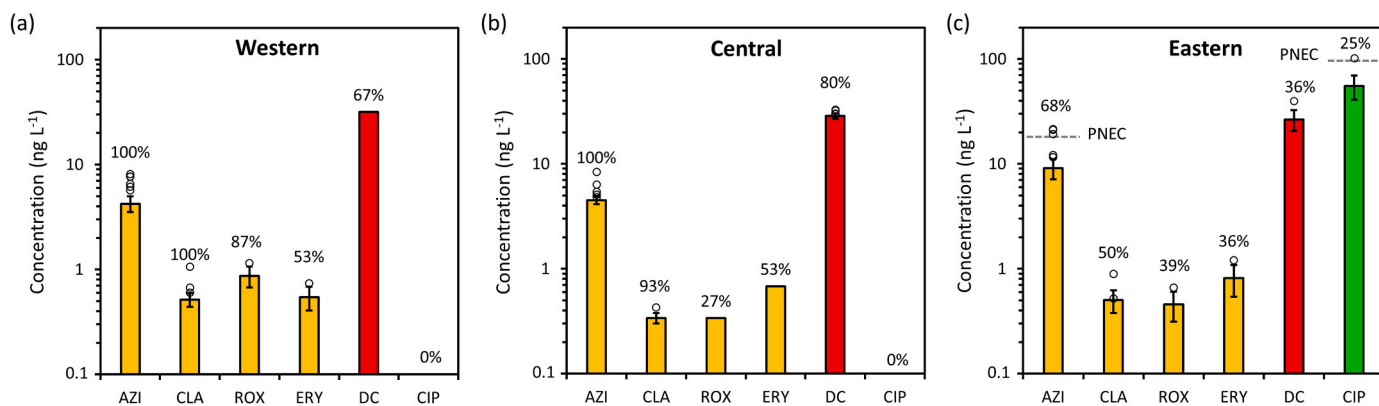


Fig. 5. The average and above-average concentrations of antibiotics detected at sites in the (a) Western, (b) Central, and (c) Eastern regions of the Chesapeake Bay. The columns are average concentrations, the circles are above-average concentrations, the error bars are standard deviation, and the labels above the columns are detection frequencies. The dashed lines indicate PNECs for azithromycin [60] and ciprofloxacin [64]. PNECs were only shown if they were exceeded by at least one measured concentration. The column colors correspond to antibiotic class: macrolide (gold); tetracycline (red); and fluoroquinolone (green). Acronyms: AZI, azithromycin; CLA, clarithromycin; ROX, roxithromycin; ERY, erythromycin; DC, doxycycline; and CIP, ciprofloxacin.

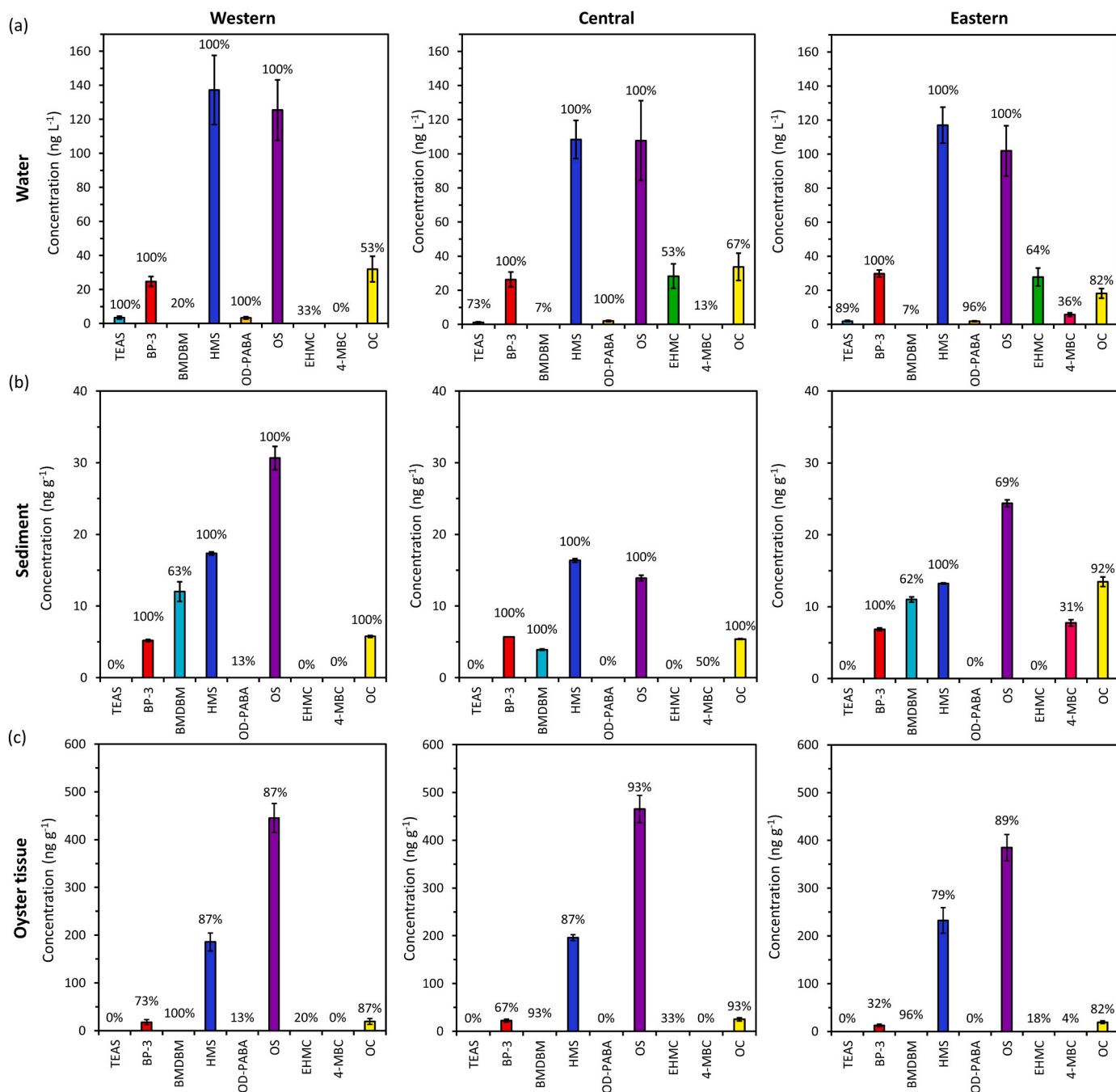


Fig. 6. Average UV filter concentrations in (a) water, (b) sediment, and (c) oyster tissue from sites located in the Western (left), Central (middle), and Eastern (right) regions of the Chesapeake Bay. The error bars are standard deviation, and the labels above the columns are detection frequencies. Acronyms: TEAS, trolamine salicylate; BP-3, oxybenzone; BMDDBM, avobenzene; HMS, homosalate; OD-PABA, padimate O; OS, octisalate; EPMC, 2-ethylhexyl 4-methoxycinnamate; 4-MBC, 4-methylbenzylidene camphor; and OC, octocrylene.

in sediment ($p = 0.52$) and oyster tissue ($p = 0.77$) did not significantly differ between regions, although oxybenzone concentrations were higher in oysters from the upper section of the Bay (Fig. 7). The results suggest the ubiquitous and pseudo-persistent occurrence of UV filters in the Chesapeake Bay, an outcome that likely stems from their incorporation into a wide variety of personal care products at high concentrations. Consider, 30 commercial sunscreens from the United States contained an average of 5.1% (v/v) oxybenzone (Table S10). Although aqueous-phase oxybenzone concentrations were similar to the $24 \pm 6 \text{ ng L}^{-1}$ reported by Gadelha et al. for sites along the Portuguese coast [78], the average oyster-phase concentration (17.6 ng g^{-1}) was much lower than that in mussels collected near Portuguese beaches

(142.7 ng g^{-1}) [79]. The apparent distribution coefficients ($K_{d,app}$) and bioaccumulation factors (BAF_{app}) were calculated for oxybenzone at sites with measurable detections in the corresponding phases (Fig. S2). In general, the $K_{d,app}$ and BAF_{app} values calculated for the Western and Eastern regions demonstrated a strong overlap, providing key knowledge of oxybenzone partitioning in the Chesapeake Bay.

Homosalate was generally detected at higher concentrations than the other UV filters in water (Fig. 6a), suggesting greater consumption and/or environmental persistence. In fact, the average homosalate concentration was $4.6 \times$ higher than that of oxybenzone. Homosalate and oxybenzone are permitted at levels of up to 15% and 6%, respectively, in sunscreen products [80]. The survey of 30 commercial sunscreens

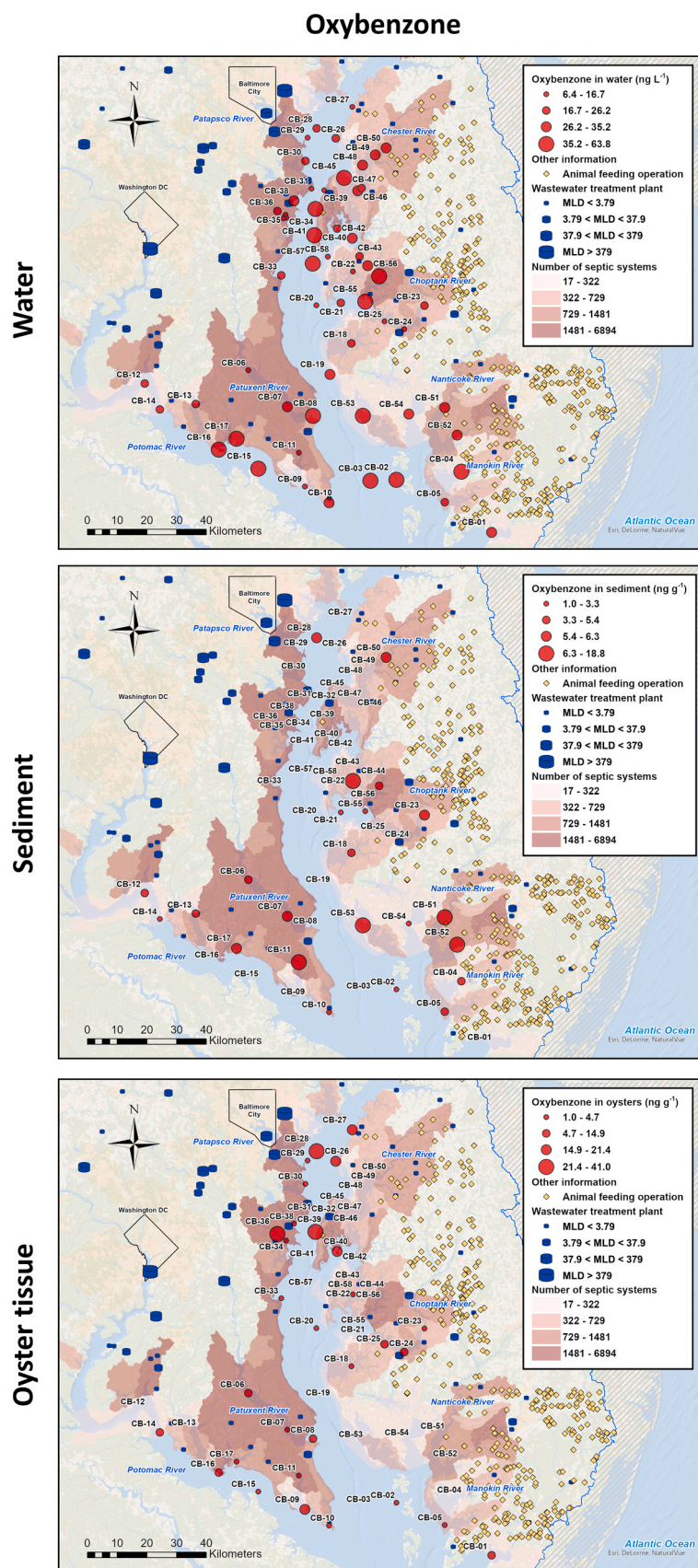


Fig. 7. Oxybenzone concentrations in water, sediment, and oysters collected from the Chesapeake Bay. The AFO [11], WWTP [12], and septic system [13] data were collected from the Maryland Department of Environment and Maryland Department of Natural Resources.

confirmed that the average homosalate content was $2.4 \times$ that of oxybenzone (Table S10). Given the concentration ratios measured in the environment, the respective levels in sunscreen products, and the high detection frequencies, the data suggest that homosalate is more persistent than oxybenzone. While Fig. 6 indicated that the average aqueous-phase homosalate concentration was higher in the Western region of the Chesapeake Bay, the difference was not significant when compared to the Central ($p = 0.41$) and Eastern ($p = 0.56$) sections, and similar conclusions were confirmed for sediment ($p = 0.64$) and oyster tissue ($p = 0.39$). These findings were reinforced by the overlap in $K_{d,app}$ and BAF_{app} values for homosalate in the Western and Eastern regions (Fig. S2). Like oxybenzone, the homosalate concentrations were higher in oysters collected from the upper section of the Bay (Fig. 8).

Although octisalate was present at similar concentrations as homosalate in water (Fig. 6), the average levels in sediment (26.1 ng g^{-1}) and oyster tissue (423 ng g^{-1}) were higher than all other UV filters. The approved limit for octisalate in sunscreen formulations is 5% [80], which is lower than that of homosalate and oxybenzone. Therefore, the relatively high concentrations of octisalate detected in water, sediment, and oysters likely stemmed from increased environmental persistence [81]. The hydrophobicity of octisalate (e.g., $\log D$ at pH 8 = 5.3) [82] likely contributed to the high concentrations observed in oysters from the upper and middle sections of the Bay (Fig. 9). Octisalate concentrations in water ($p = 0.20$), sediment ($p = 0.50$) and oysters ($p = 0.22$) were not significantly different across regions, but the $K_{d,app}$ values were generally lower in the Eastern section (Fig. S2). The reason for this outcome is unknown but may involve differences in sediment composition from the two regions.

Unlike oxybenzone, homosalate, and octisalate, higher concentrations and/or detection frequencies of 2-ethylhexyl-4-methoxycinnamate, 4-methylbenzylidene camphor, and octocrylene were detected in the Central and Eastern sections of the Chesapeake Bay. For example, the average concentrations of 2-ethylhexyl-4-methoxycinnamate were below MQL, $28.3 \pm 7.2 \text{ ng L}^{-1}$, and $27.8 \pm 5.3 \text{ ng L}^{-1}$ in the Western, Central, and Eastern regions, respectively (Fig. 6). While no explanation is proposed for these findings, it is interesting to note that (i) these three compounds are the most hydrophobic UV filters and (ii) detections were generally higher in subwatersheds with more septic systems. The maximum aqueous-phase concentration of 2-ethylhexyl-4-methoxycinnamate (71.6 ng L^{-1}) was measured at CB-50 in the Middle Chester River, which contains 3355 septic systems and three WWTPs that discharge 0.58 MLD into the immediate and upstream subwatersheds. Site CB-54 was in a subwatershed with only 294 septic systems and no upstream WWTPs, but 15 ng L^{-1} of 2-ethylhexyl-4-methoxycinnamate was detected. These data suggest septic systems were a major source of 2-ethylhexyl-4-methoxycinnamate. The 4-methylbenzylidene camphor UV filter was only detected in the Central and Eastern regions, but the detection frequencies (13–36%) and concentrations (below MQL to 7.9 ng L^{-1}) were low. In the Eastern section, octocrylene exhibited high detection frequencies in water and concentrations in sediment. In fact, the highest sediment-phase octocrylene concentration (32.9 ng g^{-1}) was found at CB-50. No significant differences were observed between regions for octocrylene levels in oysters ($p = 0.60$), but the $K_{d,app}$ and BAF_{app} values were higher in the Eastern section of the Bay (Fig. S2).

The concentrations of avobenzone, padimate O, and trolamine salicylate were lower than 10 ng L^{-1} in select samples, and only minor differences were observed between the three regions (Fig. 6). In particular, avobenzone was infrequently detected in water and oysters but regularly observed in sediment (15/23 sites), with an average concentration of 9.90 ng g^{-1} . Previous studies have reported avobenzone concentrations of $1.37\text{--}145 \text{ ng L}^{-1}$ in water from the Pearl River estuary [49] and up to 51 ng g^{-1} in sediment from the Amazon River estuary [83]. Avobenzone undergoes rapid photolysis in the environment [84], which may explain the low concentrations measured in the relatively shallow Chesapeake Bay. Padimate O (98%) and trolamine salicylate

(88%) were frequently detected in water samples, but the average concentrations were only 2.28 and 2.16 ng L^{-1} , respectively. These UV filters were also detected in some sediment (Table S8) and oyster (Table S9) samples, but no trends were apparent with respect to potential sources.

3.5. CEC sources and co-occurrence trends

The average concentrations of CECs in water, sediment, and oyster tissue were compared to geospatial data from the immediate and upstream subwatersheds to evaluate potential sources (Fig. 10). Sucralose concentrations were positively correlated to the immediate + upstream human population and number of septic systems; importantly, trends with wastewater effluent were expected but not observed. The significant correlation of sucralose concentrations to the number of septic systems is an important insight. Similarly, the trolamine salicylate (water), padimate O (water), and octisalate (oyster) concentrations were positively associated with the number of immediate + upstream septic systems, but the homosalate content in oysters was correlated to wastewater effluent in the immediate subwatershed. The only significant correlations to AFOs were the negative relationships observed for padimate O (water) and oxybenzone (oyster), suggesting that animal agriculture was not a major source of CECs across the full study area. These relationships reinforced the influence of different wastewater infrastructure on CEC concentrations in the Bay. Some previous studies in Australia and China identified trends between environmental UV filter concentrations and industrial areas with high wastewater effluent volumes [49,85]; however, the influence of septic systems on CEC concentrations has been rarely reported [32,86]. The correlations highlighted in Fig. 10 establish the importance of septic systems as primary CEC sources in the Chesapeake Bay.

Relationships between CEC concentrations in water, sediment, and oyster tissue were also evaluated by Spearman correlation to investigate co-occurrence trends (Fig. 11). Sucralose levels were positively correlated to the aqueous-phase concentrations of octocrylene, padimate O, and trolamine salicylate, confirming that these UV filters derived from septic sources. Due to the low detection frequencies of most antibiotics and hormones, co-occurrence relationships were only identified for azithromycin, which was positively correlated to the oyster tissue-phase homosalate and octisalate contents.

While most of the significant correlations between UV filters were positive, a few negative correlations were observed. For example, the aqueous-phase concentrations of oxybenzone were negatively correlated to the homosalate and octocrylene contents in oysters. The reported data inform the environmental fate of homosalate, which is understudied and poorly understood. Octocrylene is more hydrophobic than oxybenzone (Table S2) but undergoes faster degradation [87]; moreover, octocrylene is suspected to transform into oxybenzone through a retro-aldol condensation reaction [88], potentially contributing to the observed negative correlation. Azithromycin was negatively correlated to homosalate, padimate O, and trolamine salicylate concentrations in water, presumably due to the higher azithromycin levels in agriculturally influenced subwatersheds and the lack of geospatial trends for UV filters. The octisalate content in oyster tissue was negatively related to sediment-phase octocrylene concentrations, which may suggest other sources of octocrylene in agreement with Fig. 10.

Individual UV filters did not exhibit positive correlations between phases, suggesting that these CECs were not present at equilibrium at the sampling sites. For example, the octocrylene concentration in water was negatively correlated to the sediment-phase octocrylene concentration. This outcome may be due to the complex photolysis, biodegradation, partitioning, bioaccumulation, and hydrodynamic processes that govern the fate of these contaminants in the Chesapeake Bay. However, several significant positive correlations were observed between specific UV filters in one or two phases, such as homosalate and octisalate in water, sediment, and oysters (Fig. 11). The positive correlations between

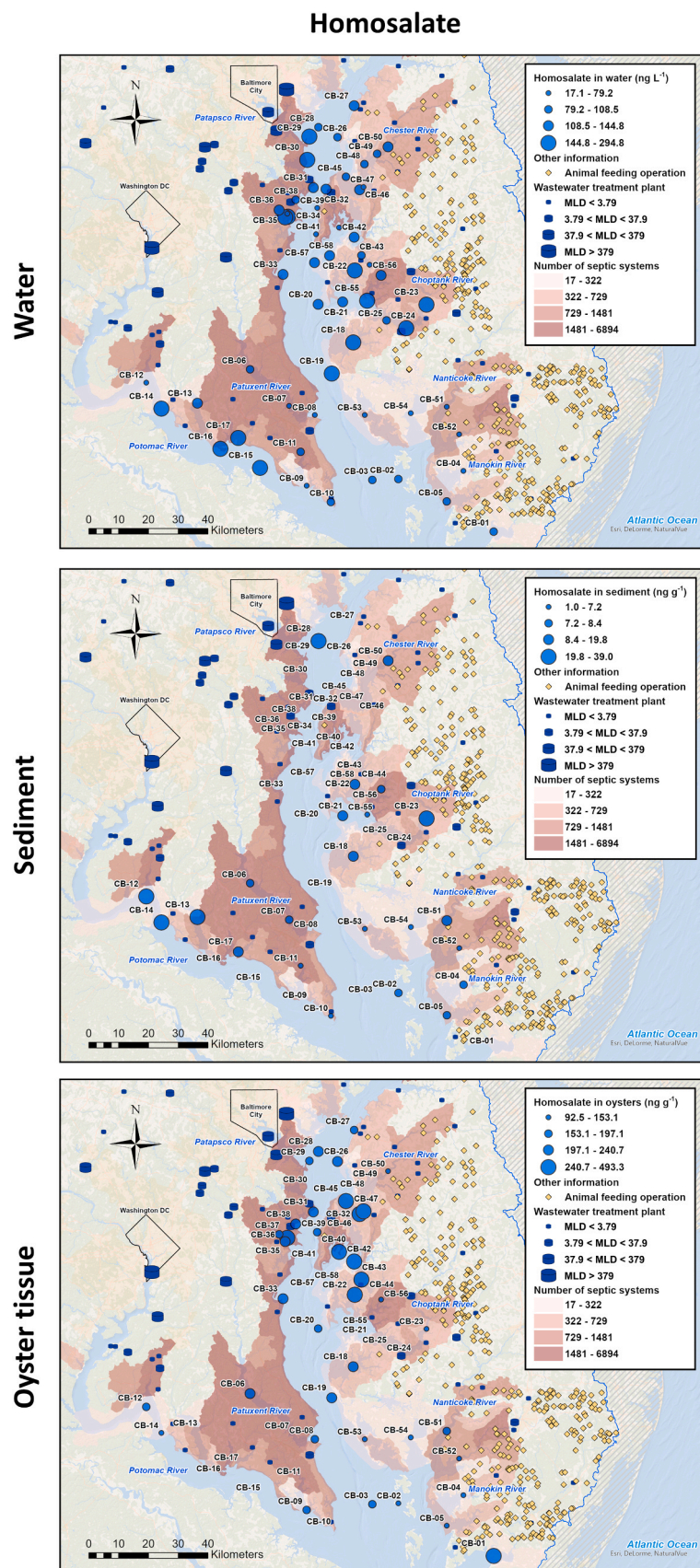


Fig. 8. Homosalate concentrations in water, sediment, and oysters collected from the Chesapeake Bay. The AFO [11], WWTP [12], and septic system [13] data were collected from the Maryland Department of Environment and Maryland Department of Natural Resources.

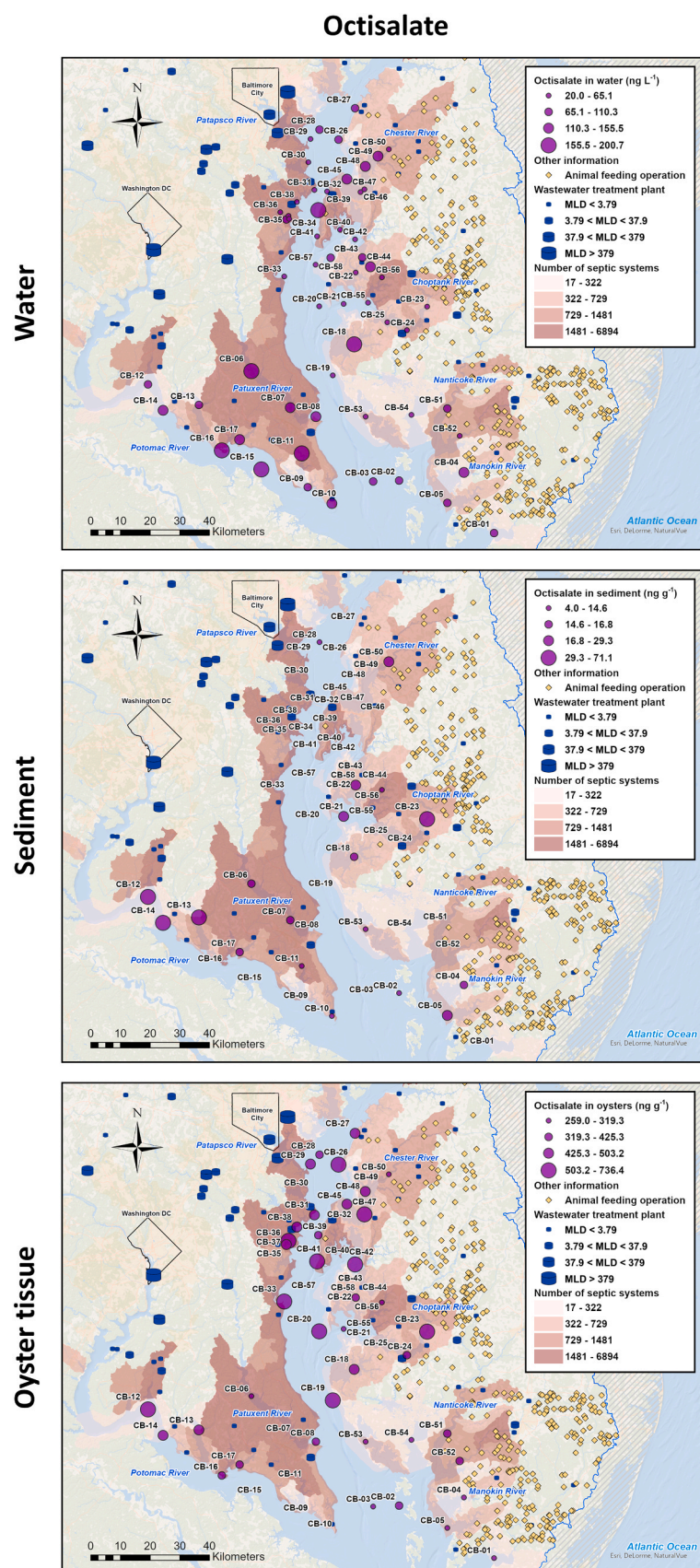


Fig. 9. Octisalate concentrations in water, sediment, and oysters collected from the Chesapeake Bay. The AFO [11], WWTP [12], and septic system [13] data were collected from the Maryland Department of Environment and Maryland Department of Natural Resources.

		Population		Septic systems		Wastewater effluent		Chickens	
		I	I+U	I	I+U	I	I+U	I	I+U
Water	AZI	0.501 0.009	0.056 0.785	-0.019 0.926	-0.139 0.500	0.427 0.029	-0.139 0.508	0.273 0.178	0.081 0.694
	CLA	-0.151 0.622	-0.206 0.499	-0.086 0.781	-0.179 0.559	-0.077 0.802	-0.161 0.617	0.386 0.193	0.149 0.628
	SUC	0.252 0.116	0.540 <0.001	0.298 0.061	0.714 <0.001	0.016 0.923	0.089 0.590	-0.132 0.416	-0.307 0.054
	BP-3	-0.368 0.015	-0.142 0.363	-0.189 0.225	-0.142 0.365	-0.004 0.980	-0.076 0.632	0.200 0.198	0.194 0.212
	OC	0.378 0.110	0.073 0.767	0.407 0.083	0.199 0.414	0.042 0.865	-0.071 0.771	0.305 0.204	0.203 0.404
	OS	0.312 0.113	0.415 0.031	0.222 0.266	0.346 0.077	0.366 0.061	0.241 0.237	-0.077 0.704	-0.146 0.468
	HMS	-0.017 0.912	0.109 0.488	0.019 0.906	-0.025 0.874	0.030 0.847	0.161 0.307	-0.182 0.243	-0.175 0.262
	OD-PADA	0.279 0.176	0.386 0.057	0.223 0.284	0.491 0.013	0.122 0.560	0.067 0.756	-0.285 0.168	-0.405 0.045
	TEAS	0.156 0.512	0.325 0.163	0.177 0.455	0.478 0.033	-0.210 0.375	-0.125 0.610	-0.364 0.114	-0.351 0.129
	EHMC	0.309 0.305	0.066 0.830	0.379 0.202	-0.083 0.788	0.171 0.577	0.427 0.146	0.260 0.391	0.358 0.229
Sediment	BP-3	0.027 0.910	-0.217 0.359	0.518 0.019	0.104 0.663	-0.179 0.450	-0.169 0.489	0.220 0.351	0.131 0.583
	OC	0.216 0.375	-0.097 0.694	-0.104 0.672	-0.284 0.238	-0.072 0.768	-0.071 0.778	-0.043 0.861	0.094 0.702
	OS	0.243 0.365	0.219 0.415	-0.121 0.655	-0.037 0.892	-0.420 0.105	0.202 0.471	-0.252 0.346	-0.018 0.948
	HMS	0.038 0.872	0.079 0.741	-0.014 0.955	0.035 0.882	-0.339 0.144	0.131 0.592	-0.016 0.947	0.035 0.883
	E1	-0.032 0.923	-0.221 0.491	0.235 0.463	0.011 0.974	0.044 0.893	-0.322 0.334	n.a.	0.108 0.739
Oyster	BP-3	-0.028 0.895	0.231 0.266	-0.027 0.898	0.256 0.216	-0.105 0.616	0.293 0.155	-0.200 0.337	-0.430 0.032
	OC	0.354 0.179	-0.322 0.225	0.492 0.053	-0.150 0.578	-0.250 0.349	-0.400 0.125	-0.074 0.785	0.142 0.599
	OS	0.376 0.053	0.595 0.001	0.031 0.878	0.451 0.018	0.308 0.118	0.411 0.033	0.061 0.761	-0.102 0.611
	HMS	0.605 0.001	0.436 0.026	0.180 0.379	0.207 0.311	0.491 0.011	0.092 0.656	0.193 0.344	-0.216 0.290

Fig. 10. Spearman correlations (top number) and significance (bottom number) between CEC concentrations in water, sediment, and oyster samples and geospatial data for subwatersheds in the immediate (I) and immediate + upstream (I + U) subwatersheds. Correlations were only evaluated for contaminants with at least 10 detections above MQL. The population, septic systems, wastewater effluent, and chickens labels correspond to the number of people, number of septic systems, wastewater effluent (in MLD), and number of chickens per subwatershed, respectively. E1 was not measured in any immediate watersheds with AFOs. The blue and red shading correspond to positive and negative correlations between the listed parameters, respectively. The bold, outlined cells indicate significant relationships. Acronyms: AZI, azithromycin; CLA, clarithromycin; SUC, sucralose; HMS, homosalate; OC, octocrylene; OD-PADA, padimate O; EHMC, 2-ethylhexyl-4-methoxycinnamate; E1, estrone; TEAS, trolamine salicylate; BP-3, oxybenzone; and OS, octisalate.

homosalate and octisalate concentrations in each phase likely stem from their similar physicochemical parameters (e.g., log D at pH 8 = 5.0 and 5.3, respectively). More importantly, the significant positive correlations reinforce the presence of common sources, especially since commercial sunscreens contain an average content (v/v) of 12.1% homosalate and 4.9% octisalate (Table S10). Given the frequent detection of UV filters in all sections of the Bay, the potential ecological risks of these CECs were investigated.

3.6. UV filters pose ecological risks to Chesapeake Bay organisms

The high detection frequencies of homosalate (86%), octisalate (91%), and octocrylene (89%) in oysters suggest that aquatic organisms are continuously exposed to UV filters and justify chronic exposure concerns. The aqueous-phase UV filter concentrations are plotted in Fig. 12 along with PNECs for Chesapeake Bay organisms. The data indicated that homosalate, octisalate, octocrylene, and oxybenzone presented potential ecological risks to select organisms. Homosalate levels were high enough to cause chronic toxicity to saltwater fish in all sections of the Bay; therefore, further studies are needed to determine the exposure and potential impacts for native fish, such as bay anchovy and white perch [89]. More than 75% of the sampling sites contained oxybenzone or homosalate concentrations that inhibit growth of the bioindicator algae *Isochrysis galbana* [41] and *Tetraselmis* spp. [42], respectively. The risks associated with oxybenzone and homosalate were lower in the Central section, which is farther downstream of potential sources and undergoes more natural attenuation. Ecological risks to photosynthetic organisms were generally similar in all regions; however, 12/15 (80%) of the Western sites contained octisalate concentrations that cause growth inhibition risks to duckweed (*Lemna gibba*) [43], compared to only 6/15 (40%) Central sites and 15/28 (54%) Eastern sites.

Chronic toxicity outcomes in daphnia (i.e., water flea) and mysid (i.e., opossum shrimp) were predicted for all regions of the Bay (Fig. 12), but greater risks were identified for 2-ethylhexyl-4-methoxycinnamate and octocrylene in the Eastern region. For example, octocrylene concentrations were high enough to cause chronic toxicity in mysid or daphnia at over 30% of the surveyed sites, including 3/15, 2/15, and 16/28 locations in the Western, Central, and Eastern sections, respectively. The measured 2-ethylhexyl-4-methoxycinnamate concentrations were capable of causing mysid mortality at the CB-49, CB-50, and CB-51 sites in the Chester River (Eastern region). Given the lower population density of Eastern region subwatersheds, the higher frequency of toxic outcomes was unexpected and warrants further investigation. Sites CB-49 and CB-50 also contained azithromycin and ciprofloxacin concentrations above their respective antimicrobial resistance PNECs (Fig. 5), highlighting the multifaceted ecological concerns at specific locations in the Chesapeake Bay, including the Chester River which was affected by WWTPs, AFOs, and septic systems.

4. Conclusion

The occurrence of CECs in matched water, sediment, and oyster samples from the Western, Central, and Eastern regions of the Chesapeake Bay was evaluated to establish contaminant profiles, identify hotspots, and determine contributions from understudied sources like AFOs and septic systems. Sucralose concentrations were positively correlated to the number of septic systems but not the volume of wastewater effluent in the immediate and upstream subwatersheds. This finding highlighted the important contribution of septic systems to CEC concentrations in the Chesapeake Bay. Macrolide antibiotics were present in 97% of water samples, but the average concentration was only 3.8 ng L⁻¹. Nevertheless, antimicrobial resistance PNECs were exceeded by azithromycin at four sites in the Chester River, and a similar result was observed for the fluoroquinolone antibiotic, ciprofloxacin, at one site in the same river. Hormones were only detected in 2% of water and

	Water								Sediment				Oyster				
Water	SUC																
	-0.022 0.894	AZI															
	-0.088 0.524	-0.017 0.918	BP-3														
	0.026 0.849	-0.387 0.014	-0.175 0.190	HMS													
	0.625 0.004	-0.071 0.867	-0.202 0.368	-0.048 0.832	OC												
	0.449 0.010	-0.494 0.017	-0.178 0.329	0.150 0.413	0.469 0.145	OD-PABA											
	0.082 0.665	-0.265 0.272	0.250 0.161	0.359 0.040	0.442 0.114	0.369 0.100	OS										
	0.484 0.012	-0.609 0.007	-0.289 0.153	0.360 0.070	0.354 0.215	0.877 < 0.001	0.384 0.142	TEAS									
Sediment	-0.036 0.887	-0.480 0.160	-0.173 0.454	-0.223 0.330	-0.148 0.629	-0.322 0.398	-0.257 0.355	-0.200 0.555	BP-3								
	0.397 0.083	0.551 0.079	-0.368 0.101	0.200 0.385	0.245 0.467	0.064 0.851	-0.359 0.188	0.095 0.770	0.252 0.298	HMS							
	-0.474 0.040	0.487 0.128	0.280 0.232	0.134 0.574	-0.721 0.019	-0.135 0.709	-0.411 0.144	-0.223 0.509	-0.061 0.810	0.073 0.767	OC						
	0.208 0.422	0.542 0.085	-0.203 0.434	0.326 0.202	-0.536 0.215	0.037 0.919	-0.253 0.405	0.310 0.354	-0.239 0.390	0.710 0.001	0.375 0.138	OS					
Oyster	0.033 0.850	0.580 0.002	-0.329 0.044	0.107 0.521	0.512 0.036	0.327 0.128	0.155 0.490	0.226 0.338	0.411 0.128	0.143 0.626	-0.088 0.775	0.009 0.979	HMS				
	-0.189 0.367	0.108 0.680	-0.520 0.006	-0.169 0.409	0.377 0.283	-0.115 0.696	-0.275 0.341	-0.090 0.781	0.750 0.020	0.048 0.911	-0.017 0.966	-0.314 0.544	0.352 0.139	OC			
	0.300 0.072	0.548 0.004	-0.311 0.051	0.082 0.615	0.362 0.140	0.362 0.107	0.183 0.391	0.114 0.623	0.105 0.677	0.709 0.001	-0.662 0.005	0.385 0.175	0.492 0.004	0.161 0.498	OS		

Fig. 11. Spearman correlations between CEC concentrations in water, sediment, and oyster tissue. Correlations were only evaluated for contaminants with at least 10 detections above MQL, and CECs without any significant relationships were omitted from the figure. The blue and red shading correspond to positive and negative correlations between the listed parameters, respectively. The bold, outlined cells indicate significant relationships. Acronyms: SUC, sucralose; AZI, azithromycin; BP-3, oxybenzone; HMS, homosalate; OC, octocrylene; OD-PABA, padimate O; OS, octisalate; and TEAS, trolamine salicylate.

37% of oyster samples, but the high detection frequencies (74%) in sediment were associated with subwatersheds that contained many septic systems. While their concentrations did not exhibit major differences across the Western, Central, and Eastern regions of the Bay, several UV filters were associated with population, wastewater effluent, and/or the number of septic systems in the immediate and upstream subwatersheds. Homosalate was predicted to confer ecotoxicological outcomes to saltwater fish at all studied sites. Overall, this study provided a comprehensive set of CEC data for water, sediment, and oysters in a critical United States estuary, highlighted the importance of

wastewater, septic system, and AFO sources at different sites in the watershed, and identified chronic toxicity and antimicrobial resistance concerns from UV filters throughout the Chesapeake Bay and antibiotics in the Chester River, respectively.

Environmental Implications

The environmental occurrence of antibiotics leads to development of antimicrobial resistance. Hormones and UV filters cause endocrine disrupting effects that harm aquatic organisms. These contaminants of

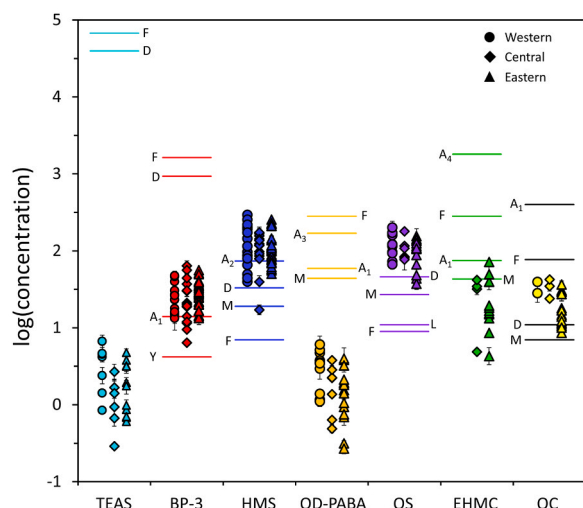


Fig. 12. The log-based concentrations of trolamine salicylate (TEAS), oxybenzone (BP-3), homosalate (HMS), padimate O (OD-PABA), octisalate (OS), 2-ethylhexyl-4-methoxycinnamate (EHMC), and octocrylene (OC) overlaid with PNECs for aquatic organisms relevant to Chesapeake Bay ecology. All concentrations are in units of ng L^{-1} . Measured concentrations are only shown if they were above the MQL, and the total number of measurements in the Western, Central, and Eastern regions were 15, 15, and 28. The horizontal lines indicate PNECs derived from toxicity data for (A₁) *I. galbana* [41,45], (A₂) *Tetraselmis* spp. [42], (A₃) *Scenedesmus vacuolatus* [46], (A₄) *Raphidocelis subcapitata* [47], (D) daphnid [43], (F) saltwater fish [43], (L) *Lemna gibba* [43], (M) mysid [43], and (Y) the yeast estrogen screen [44]. The specific toxicity thresholds and endpoints are provided in Table S6.

emerging concern present a global health challenge and are considered hazardous materials. This work constitutes the first effort to measure water-, sediment-, and oyster-phase concentrations of a large suite of contaminants of emerging concern throughout the Chesapeake Bay (USA) and to connect those concentrations to geospatial data from upstream subwatersheds. Correlations between contaminant concentrations and geospatial data were established to identify potential sources, including septic systems, which have been understudied in prior literature.

CRedit authorship contribution statement

Ethan Hain: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Ke He:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation. **Jahir A. Batista Andrade:** Data curation, Formal analysis, Methodology, Validation, Visualization. **Anna Feerick:** Investigation. **Mitchell Tarnowski:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources. **Anne Timm:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology. **Lee Blaney:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Resources, Writing – original draft, Writing – review & editing, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All data are provided in the supplementary material.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132405.

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